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# A new, genetically divergent species of *Pseudobaikalia* Lindholm, 1909 (Caenogastropoda, Baicaliidae)

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## Abstract

A new gastropod species, *Pseudobaikalia michelae* Sitnikoiva & Kovalenkova, **sp. n.**, (family Baicaliidae) is described from Lake Baikal. This is the first new species from the Baicaliidae for forty years. The new species is distinguished from its sister taxa by means of comparative morphology as well as analyses of DNA sequences (mtDNA and an intron of alpha-subunit gene of ATP-synthase). It was found in the southern and central-eastern parts of the lake where it occurs sympatrically with three other baicaliid species. Characters of the female reproductive system (i.e., a long oviduct loop with 2–3 narrow tube-like evaginations) and the aperture (i.e., oval shape with a simple outer lip) place these snails in the genus *Pseudobaikalia* Lindholm, 1909. The new species is most similar in its shell morphology to the northern Baikal species *P. jentteriana* (smooth elongated shape) but differs by a more oval aperture that is slightly angled to the columella. Combined mitochondrial and nuclear sequences in a Bayesian analysis showed that all specimens of *P. michelae* **sp. n.** form a well-supported clade.

## Keywords

Gastropoda, new species, *Pseudobaikalia michelae*, Lake Baikal, Siberia

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## Introduction

Lake Baikal is the most ancient freshwater lake on Earth. It is inhabited by very diverse endemic species flocks (Sherbakov 1999). The family Baicaliidae Fisher, 1885 (Radoman 1983, Sitnikova et al. 2004) or subfamily Baicaliinae of the family Amnicolidae (Bouchet et al. 2005, Kantor et al. 2010) includes eight genera and nearly 40 species (Dybowski 1875, Lindholm 1909, Kozhov 1936, Sitnikova et al. 2004). The last new species description for this taxon dates back to 1975 (Beckman and Starobogatov 1975), more than 40 years ago. At present, nucleotide sequences for mitochondrial and nuclear genes are known for 23 species of baicaliids (Zubakov et al. 1997, Peretolchina et al. 2008, Kovalenkova et al. 2015). Molecular analyses of baicaliids have revealed limited phylogenetic structure based on mtDNA sequences (Zubakov et al. 1997). In this study the phylogeny inferred from sequences of cytochrome c oxidase subunit I (CO1), mitochondrial small subunit rDNA (mtSSU) and intron of alpha-subunit gene of ATP-synthase confirms that specimens of *Pseudobaikalia michelae* sp. n. form a separate clade. Indeed, unique conchological and anatomical characters of these snails add support to their status as a new species.

## Material and methods

The type material of *Pseudobaikalia michelae* sp. n. was collected by dredging during sampling expedition to Lake Baikal on 11 October 2009. Additionally, two specimens of the new species were defined among syntypes of *Pseudobaikalia pulla pulla* (=*Leucosia angarensis* var. *pulla* W. Dybowski, 1875) collected by Benedict Dybowski, probably near Kultuk settlement, place of his political exile, and hosted in the freshwater gastropod collection of Zoological Institute RAS (Saint Petersburg). Some snails were found at three further sites of the Lake (Fig. 1) and stored in Limnological Institute SB RAS (Irkutsk).

The representatives of other species of baicaliids used for molecular analyses were collected at different sites of the lake during expeditions in 2009–2014 by dredging or diving (for details see Table 1). The shells of the new species were compared to type specimens of baicaliids (Fig. 2D–K) housed in the collection of the Zoological Institute RAS.

Anatomical study and molecular analysis were performed using snails initially fixed in 80% ethanol and then stored in 70% ethanol after one day. Seven snails were photographed and then shells of six individuals were destroyed for dissection. Micrographs of protoconchs and radulae were produced using a SEM while soft tissues were dissected under a light stereomicroscope. Morphological study and descriptive terminology are based on the review of morphological characters of hydrobioid gastropods (Radoman 1983, Hershler and Ponder 1998). Measurements of the shells, radular teeth and inner organs were performed using the Image-Pro-Plus program for Windows XP.

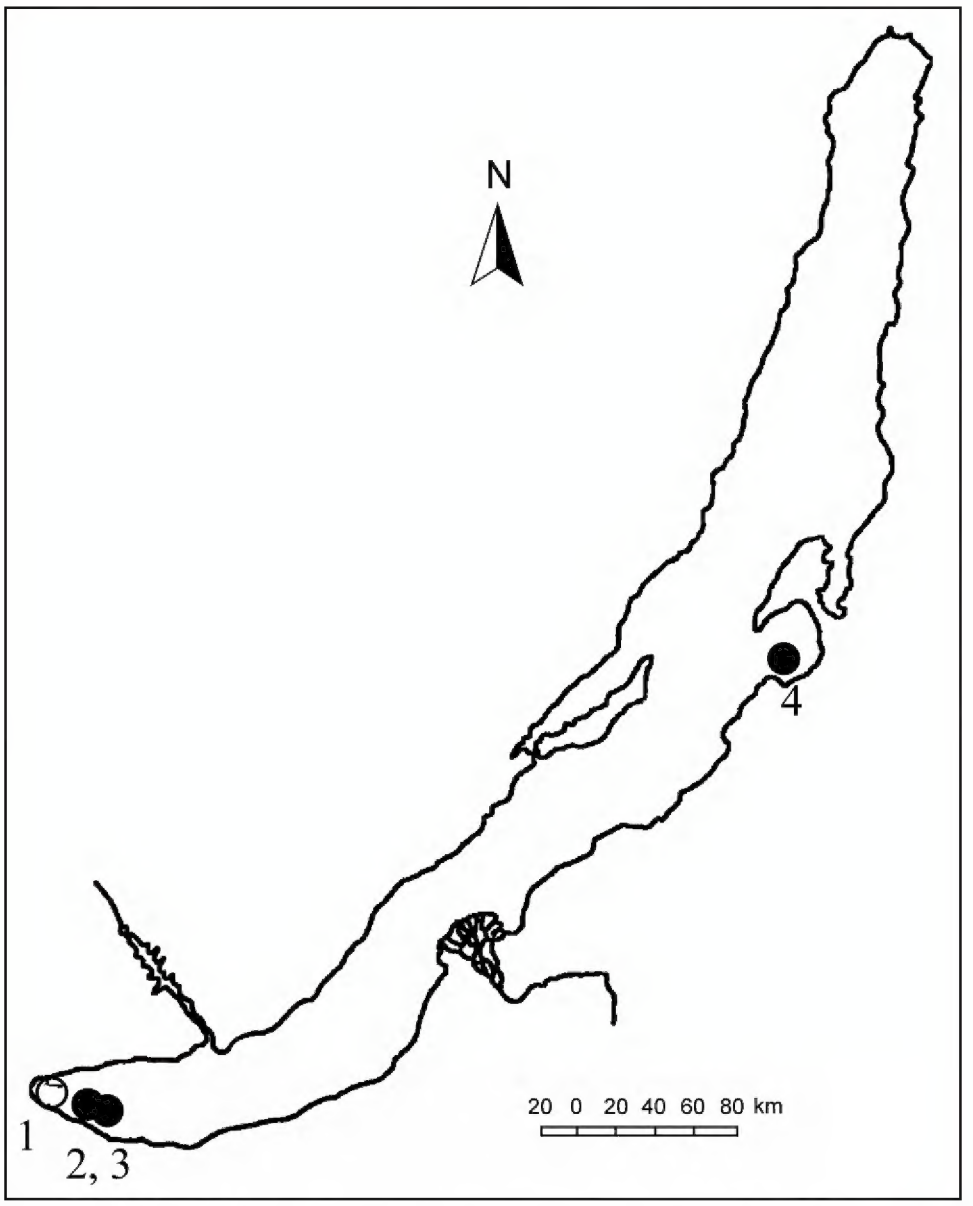


**Figure 1.** The location of *Pseudobaikalia michelae* sp. n. sampling sites in Lake Baikal. Site numbers correspond to **1** Kultuk Bay **2** near Utulik settlement **3** Murinskaya Bank **4** Barguzin Bay.

DNA was extracted from muscle tissue from the molluscan foot by using the modified method of Doyle (Doyle and Doyle 1990). We used universal pairs of primers for amplification and sequencing fragments of the mitochondrial genes CO1 (Folmer et al. 1994), and mtSSU (Katana et al. 2001), as well as segment of the nuclear gene of ATP synthetase alpha-subunit containing a single intron (ATPase α) (Jarman et al. 2002). Polymerase chain reaction (PCR) amplifications (35 cycles) were carried out using a BioRad T100 Thermal Cycler under the following conditions: pre-denaturation of DNA at 94 °C for 2 min, denaturation of DNA at 94 °C for 20 s, primer annealing 1 min at 48 °C for CO1, 50 °C for mtSSU rDNA and 54 °C for intron, and nucleotide chain elongation at 72 °C for 1 min (+ 5 min in the last cycle). The amplification products were sequenced at JSC Sintol (Moscow). The nucleotide non-coding sequences were aligned taking into account their putative secondary structures using Mafft v. 6 (Katoh et al. 2009).

Localities and GenBank accession numbers for sequenced species are given in Table 1.

**Table 1.** Collection localities and GenBank accession numbers for specimens included in the molecular analysis; n=number of specimens.

| Taxon | Locality, depth in meters, substrate (collection date) | GenBank Accession No. | | |
|---|---|---|---|---|
| | | CO1 | 16S rDNA | Intron ATP α |
| *Parabaikalia elata* (n=2) | Peschanaya Bay, 8.6 m, sand (29.07.09) | KT885122 | – | KF201704 |
| *Pseudobaikalia contabulata* (n=2) | Kurkut Bay, 5 m, sand (27.08.11); Olkhon Gate, 15 m, sand (03.09.12) | KT808643, KT808642 | KT885135, KT885136 | KT885109, KT885134 |
| *Pseudobaikalia jentteriana* (n=2) | Olkhon Gate, 37–38 m, sand, silt (13.10.09) | KT808645, KT885125 | KT885137, KT885138 | KT885104, KT885105 |
| *Pseudobaikalia michelae* sp. n. (n=6) | Kultuk, 11–27 m, stones, coarse sand (11.10.09) | KT808639 – KT808641, KT885126 – KT885128 | KT885139 – KT885144 | KT885096 – KT885101 |
| *Pseudobaikalia pulla pulla* (n=2) | Listvyanka, 10–14 m, sand, silt (06.02.14) | KT885129 | KT885145 | KT885107 |
| *Pseudobaikalia pulla tenuicosta* (n=3) | Olkhon Gate, 37–38 m, sand, silt (13.10.09); Onokachanskaya, 10–15 m, sand (24.09.13) | KT808646, KT808648 | KT885146, KT885147 | KF201700, KT885108 |
| *Pseudobaikalia zachwatkini* (n=2) | Listvyanka, 10–14 m, sand, silt (06.02.14) | KT885130 | KT885148 | KT885095 |

Sites of mtSSU rDNA and ATPase α containing indels were excluded from the analysis. Concatenated 1529 bp long fragments (618 bp of mtDNA CO1, 445 bp of mtSSU rDNA, and 466 ATPase α) were used for phylogenetic inferences despite the lack of ATPase α data for the outgroup taxa. mtSSU rDNA data was also missing for *Parabaikalia elata*. *Benedictia baicalensis* (Benedictiidae: Caenogastropoda) was used as the closest available outgroup based on a previous phylogenetic analysis (Hausdorf et al. 2003), although the substitutions at the third codon position in CO1 may potentially impede the inferences. The nucleotide sequences of CO1 and mtSSU rDNA for *B. baicalensis* were taken from GenBank (accession numbers AF445330 and AF445339).

JModelTest v. 2.0.2 (Guindon and Gascuel 2003, Darriba et al. 2012) was used to find the appropriate substitution model by means of the Bayesian Information Criterion. The phylogenetic tree was inferred by the Bayesian total evidence method as implemented in MrBayes 3.2.1 (Ronquist et al. 2012). Four replicate analyses of four simultaneous chains (1 cold) were run for 6,000,000 generations, sampling trees every 100 generations; the first 30% of trees were discarded. Trees sampled from the stationary phase of each replicate analysis were pooled to construct a single 50% majority rule consensus tree with Bayesian posterior probabilities. Uncorrected genetic distances (*p*-distances) were computed in the package APE (Paradis et al. 2004) using R (R Core Team 2015).

## Results

### Taxonomy

### Family BAICALIIDAE Fisher, 1885

### Genus *PSEUDOBAIKALIA* Lindholm, 1909

*Baikalia* (*Pseudobaikalia*) Lindholm, 1909: 42. Type-species: *Baikalia jentteriana* 1909 (by original designation).
*Baicalia* (*Pseudobaicalia*): Kozhov 1936: 85 (type species *B. contabulata* Dybowski, 1875).
*Pseudobaikalia*: Sitnikova 1991: 285 (female reproductive system morphology); Sitnikova et al. 2004: 947 (type species *Baicalia jentteriana*, species composition); Kantor et al. 2010: 28 (type species *Baikalia jentteriana*, list of species).

**Diagnosis.** Shell elongated, height up to 10 mm at 5–6 well rounded or shouldered whorls, smooth or with transverse fine ribs, oval aperture with simple evenly rounded outer lip, without umbilicus, protoconch discoidal, lateral radular teeth with square face, its width equal to length of outer wing; length of capsule gland equal to albumen gland, loop of oviduct long, reaching the proximal end of albumen gland, oviduct loop a cluster that includes from 2–7 'tube-like evaginations', sometimes beyond the albumen gland.

**Remarks.** The earlier diagnosis of genus (subgenus) *Pseudobaikalia* involved only conchiological traits (Lindholm 1909, Kozhov 1936) or morphology of reproductive system (Sitnikova 1991). Presently, the morphological details obtained from our study of *P. michelae* sp. n. and early published data on radular teeth (Kozhov 1936) and protoconchs (Sitnikova et al. 2001) conform to the emended diagnosis of the genus *Pseudobaikalia*. Besides *P. michelae* sp. n. the genus includes *B. jentteriana*, Lindholm, 1909, *B. contabulata* (Dybowski, 1875), *B. pulla pulla* (Dybowski, 1875) (= *B. subcilindrica* Lindholm, 1909), *P. pulla tenuicosta* (Lindholm, 1909), *P. elegantula* (Lindholm, 1909), *P. cancellata* (Lindholm, 1909), and *P. zachwatkini* (Kozhov, 1936). The shell photos of the types of these earlier described species are presented here for the first time (Fig. 2D–K).

### *Pseudobaikalia michelae* Sitnikoiva & Kovalenkova, sp. n.

http://zoobank.org/547D6538-64A8-4E90-8503-3048991BB626
Figs 2A, B, C, 3–5

**Etymology.** The species name '*michelae*' is in honour of Ellinor Michel (Natural History Museum, London) who has made a range of studies on gastropods inhabiting ancient lakes.

**Type material.** Holotype (dry) and 2 paratypes (dry and in alcohol) from the type locality were deposited in freshwater gastropod collection of Zoological Institute

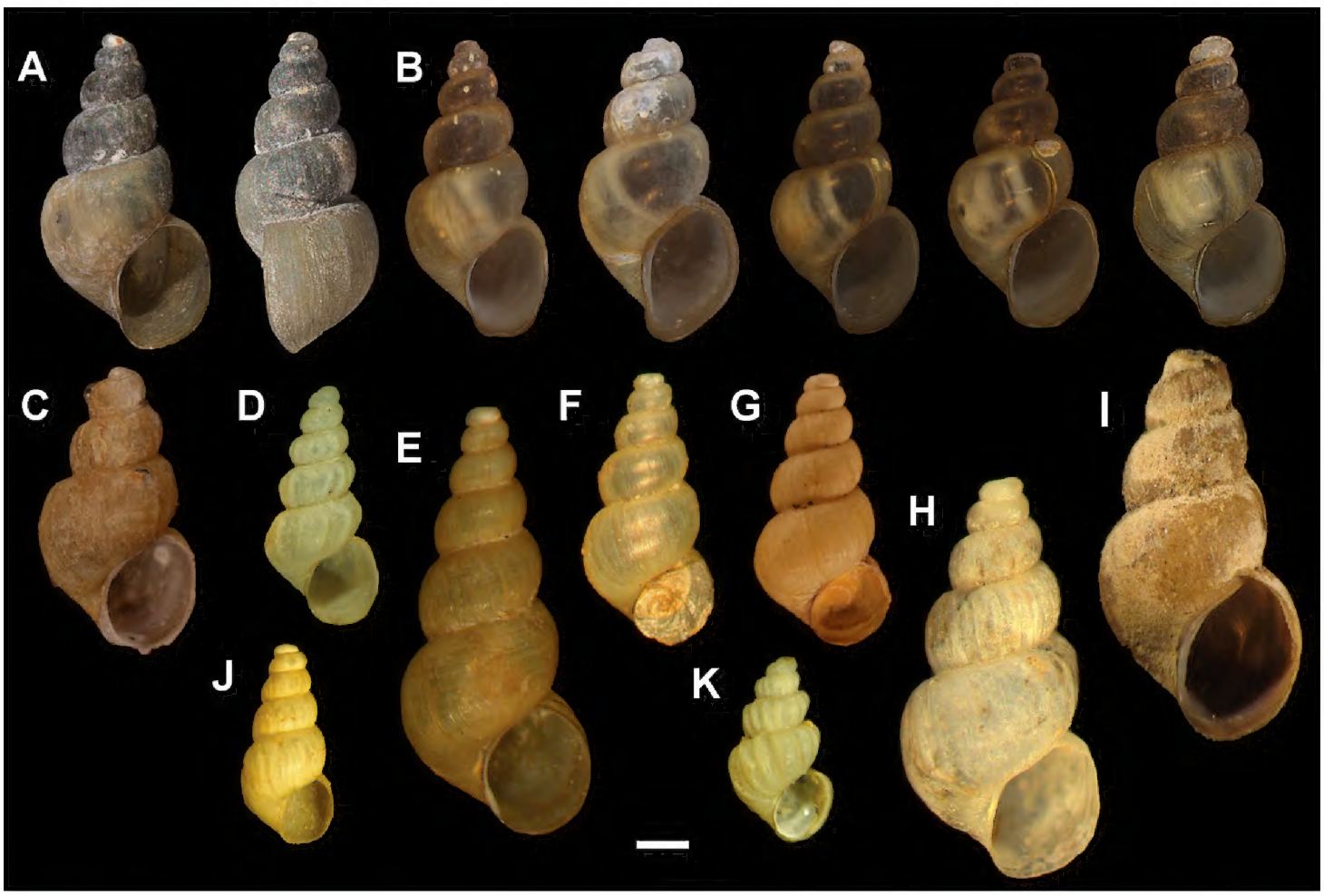


**Figure 2.** Shells of the type specimens of *Pseudobaikalia michelae* sp. n. and syntypes of other species of *Pseudobaikalia* genus from ZIN collection. **A** Holotype *P. michelae* sp. n. **B** Paratypes *P. michelae* sp. n. used for analyses **C** *P. michelae* sp. n. (=*Leucosia angarensis* var. *pulla*, det. Dybowski, No. 1) **D** *P. elegantula* (No. 2) **E** *P. jentteriana* (No. 1) **F** *P. pulla pulla* (No. 1) **G** *P. p. tenuicosta* (No.1) **H** *P. zachwatkini* (No. 1) **I** *P. elata* (=*B. angarensis elata*, No.1) **J** *P. cancellata* (No. 1) **K** *P. contabulata* (No. 1). Scale bar 1 mm.

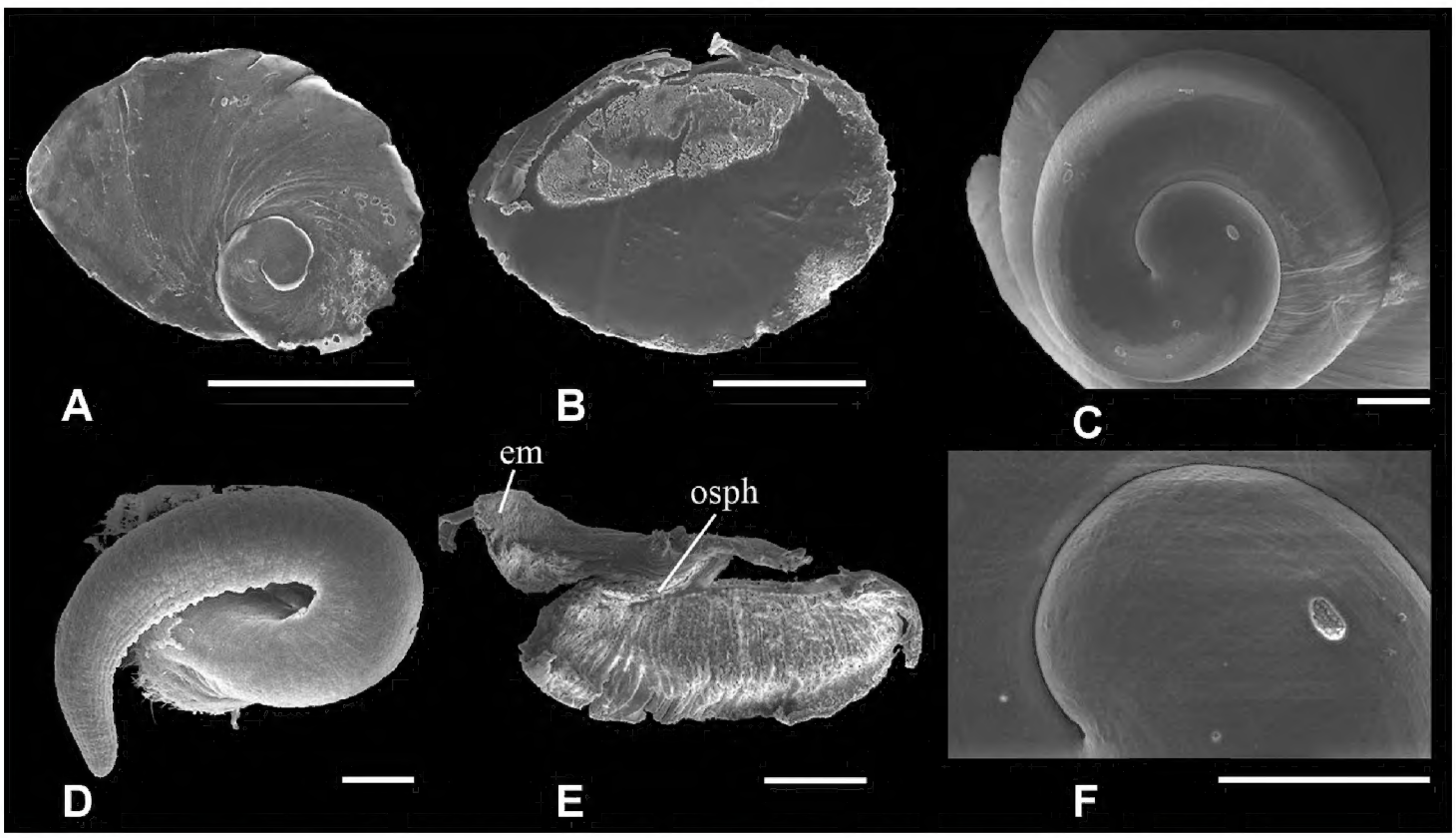


**Figure 3.** Scanning electron micrographs of *Pseudobaikalia michelae* sp. n. **A, B** Operculum, dorsal view to left and ventral view to right **C, E** Protoconch **D** Penis, dorsal view. Scale bars: 0.5 mm (**A, B**); 0.1 mm (**C, D, E**).

RAS (Saint Petersburg, Russia) and are registered under Nos. 1/522–2015 (holotype) and 2/522–2015 (paratypes). Shells of six paratypes were destroyed for dissections (to study anatomy and nucleotide sequences) with images of them given here (Fig. 2B).

**Additional material.** Two shells from a lot No. 1 of Zoological Institute collection with original label '*Leucosia angarensis* var. *pulla*, Baikal, collection W. Dybowski 1875'; 2 specimens: south-eastern part of Lake Baikal, near Utulik settlement (51°33'13.5"N, 104°05'17.3"E), at the depth of 17–31 m, silty sand; by drag, coll. by authors 07.21.2015 (site 2); 2 shells: the same part of the lake, Murinskaya Bank (51°30'11.3"N, 104°28'39.4"E), 34–39 m depth, stones, sand, silt; by drag, coll. by authors 07.21.2015 (site 3); 7 specimens: central part of the Lake, Barguzin Bay (53°18'34.8"N, 108°44'07.4"E), at a depth of 14 m, sand and stones; by drag, coll. by authors 07.17.2015 (site 4 in Fig. 1).

**Type locality.** Kultuk Bay (southern part) of the Lake Baikal (southern Siberia), (51°42'59.9"N, 103°43'23.1"E) from 11 to 27 m depth, sand and stones, sponges (site 1, Fig. 1).

**Description.** (Figs 2–5): Shell (Fig. 2A, B) grey-green or light brown, elongate-conic, smooth with 5–6 growth lines, small, up to 6.5 mm high and 3 mm wide; with 4.25–5.75 well rounded whorls, deep suture, without umbilicus; aperture elongate-oval, columellar lip slightly thickened, outer lip thin, simple or slightly rounded, basal lip rounded or slightly elongated. For shell dimensions please see Table 2. Protoconch (Fig. 3C, F) discoidal, about 1.25–1.45 whorls, diameter about 500 μ, surface almost smooth, near suture with slight spiral threads or slightly reticulate. Operculum (Fig. 3A, B) flat, thin, transparent, paucispiral with 5–6 growth whorls, last half whorl weakly frilled, nucleus subcentral, attachment scar elongated oval occupies about 1/3 of operculum width.

Radula (Fig. 4): 580–600 μm length with 46–48 teeth rows, 30–33 of them well-formed. Central teeth square or triangular, about 15.5 μm wide, cutting edge broadly concave; central cusp absent or square formed by the merger of 2–3 cusps; lateral cusps 15–16, thin and long; basal tongue slightly convex or straight. Lateral teeth face rectangular, outer margin with concave bend, central cusp similar to lateral cusps or slightly broader, or merger of 2–3 cusps, inner and outer lateral cusps about 9–10; outer wing rather broad, straight, about two times longer than cutting edge. Inner marginal teeth with approximately 24 cusps and outer marginal teeth with approximately 16 cusps.

Body pigmented black, mantle edge light grey, ctenidium nearly 2 mm in length with 62–64 leaflets, osphradium broadly ovate, slightly narrowed in proximal end, ~ 0.5 mm length, opposite anterior part of ctenidium, a little deeper than mantle fold.

**Reproductive system.** Male with small bean prostate gland (Figs 5D, 3D), anterior and posterior vas deferens close together in middle part of prostate, penis light grey, muscular, elongated, with thin glandulous often non-visible fold, gradually tapering with short and small papilla. Female coiled oviduct with one spiral, loop grey pigmented with two 'tube-like evaginations' (Fig. 4A–C).

**Ecology.** *P. michelae* sp. n. was found on heterogeneous (stones, sand, and silt) or soft sediments at depth zone ranging from 11 to 39 m in southern, east-southern and

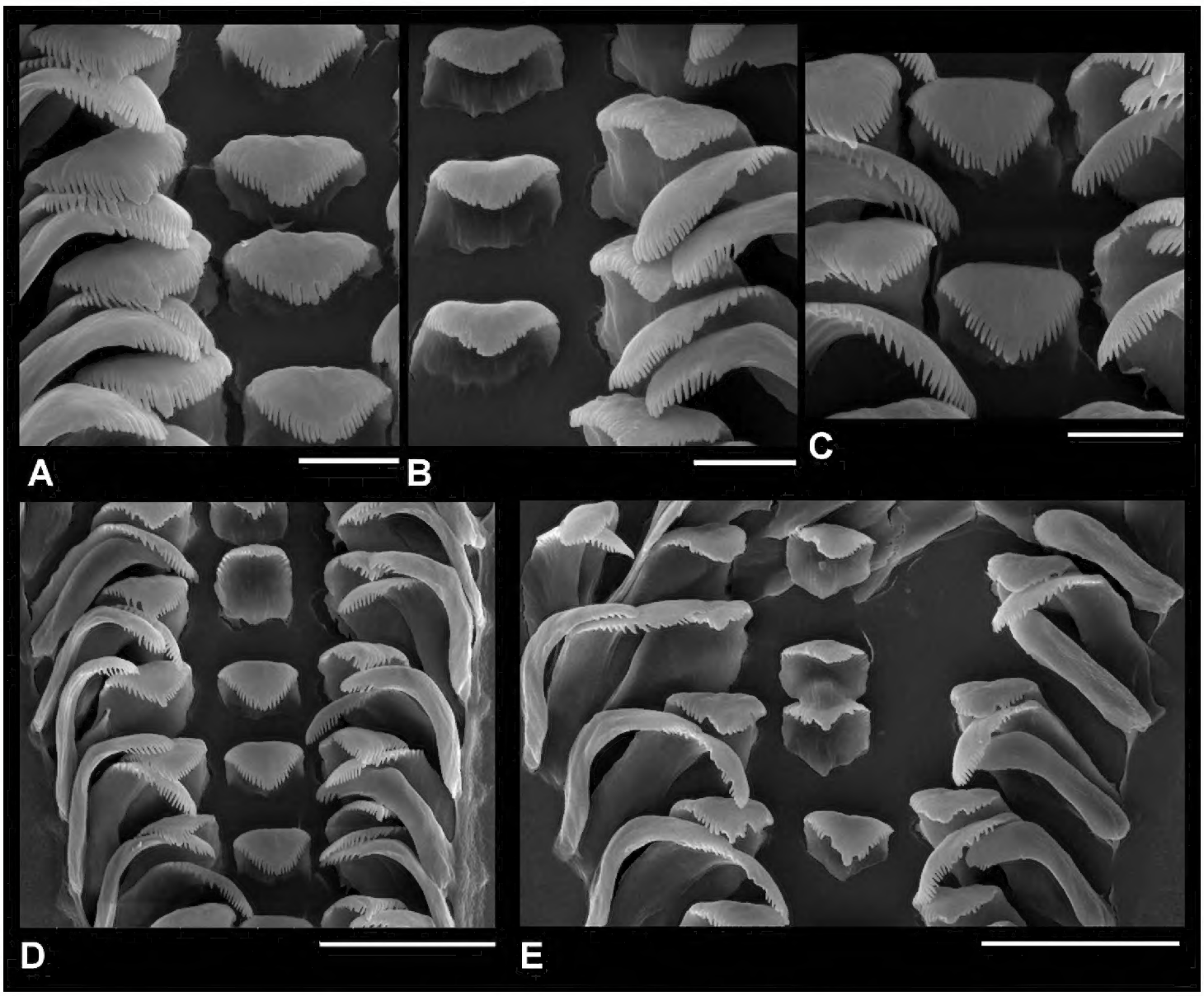


**Figure 4.** Radular teeth of *Pseudobaikalia michelae* sp. n. Scale bars: 10 μm (**A, B, C**); 30 μm (**D, E**).

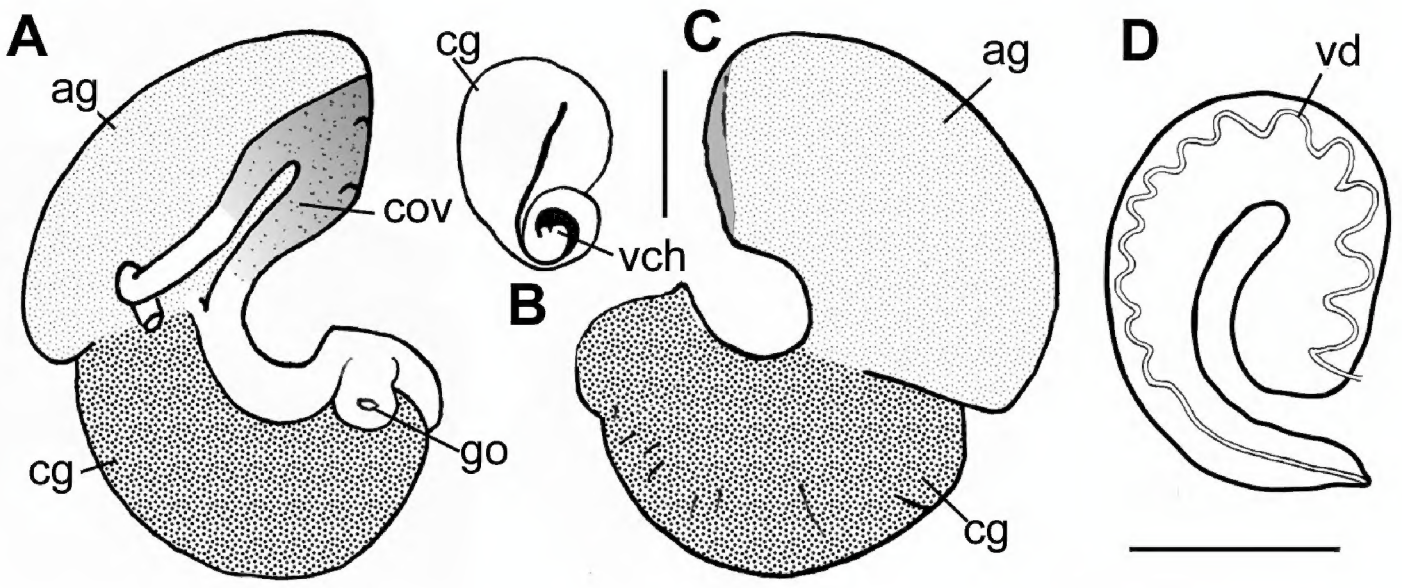


**Figure 5.** Genitalia of *Pseudobaikalia michelae* sp. n. **A, C** Ventral and dorsal view of female genitalia **B** Section of capsule gland and ventral channel **D** Penis. Abbreviations: ag—albumen gland; cg—capsule gland; cov—coil oviduct; go—genitalia opening; vch—ventral channel; vd—vas deference. Scale bars 0.5 mm.

**Table 2.** Shell dimensions and whorl counts of type material of *Pseudobaikalia michelae* sp. n. Abbreviation: SH—height of shell; SW—width of shell; AW—width of aperture; AL—length of aperture; SpH—height of spire; LW—height of last whorl; n—number of whorls. Measurements are in mm.

| Specimen | SH | SW | AW | AL | SpH | LW | n |
|---|---|---|---|---|---|---|---|
| Holotype | 6.33 | 3.40 | 1.90 | 2.60 | 2.13 | 4.20 | 5.5 |
| Paratypes | 5.72 | 3.04 | 1.65 | 2.52 | 1.55 | 4.17 | 4.5 |
| | 5.98 | 2.84 | 1.62 | 2.16 | 2.12 | 3.86 | 5.75 |
| | 5.64 | 2.86 | 1.61 | 2.32 | 1.93 | 3.71 | 4.75 |
| | 5.08 | 2.82 | 1.62 | 2.25 | 1.45 | 3.63 | 4.25 |
| | 5.70 | 2.93 | 1.71 | 2.26 | 1.90 | 3.80 | 4.5 |
| | 5.06 | 2.64 | 1.49 | 2.03 | 1.70 | 3.36 | 5 |
| | 4.87 | 2.88 | 1.58 | 1.85 | 1.67 | 3.20 | 3.75 |
| Mean ± SD (n = 7) | 5.64 ± 0.46 | 2.93 ± 0.24 | 1.66 ± 0.13 | 2.31 ± 0.20 | 1.83 ± 0.27 | 3.82 ± 0.30 | 5.05 ± 0.5 |
| Shell from ZIN collection | 5.41 | 2.95 | 1.64 | 2.23 | 1.69 | 3.72 | (3.5) |

central-eastern parts of Lake Baikal. At the type locality *P. michelae* sp. n. was sympatric with three other species of baicaliids: *Godlewskia pulchella* (Dybowski, 1875), *Korotnewia semenkewitschi* Lindholm, 1909, and *Teratobaikalia duthiersii* Dybowski, 1875. Eleven baicaliids including *P. michelae* sp. n. and three species of the genus *Pseudobaikalia*: *P. contabulata*, *P. pulla*, and *P. zachwatkini* were collected together at sites 2 and 4 (Fig. 1); ten baicaliids were registered at site 3 (Fig. 1), including the species, *P. contabulata* and *P. zachwatkini*.

**Remarks.** The genus *Pseudobaikalia* includes seven mainly shallow water species (Fig. 2D–K); some of them were found down to 100 or 200 m depth. Two species *P. jentteriana* and *P. cancellata* were found only in the northern part of the lake, *P. elegantula* inhabits the northern and central parts of the lake, and three other species *P. zachwatkini*, *P. pulla*, and *P. contabulata* are found in a range of locations throughout of Baikal, and are sympatric to *P. michelae* sp. n.

The shells of *P. jentteriana* (Fig. 2E) and *P. pulla pulla* (Fig. 2F) are smooth as in the new species, while the other *Pseudobaikalia* have transverse ribs or lirae. The shell of *P. pulla tenuicosta* (from the northern part of Lake Baikal) has slightly raised ribs. Both subspecies of *P. pulla* have an operculum equal to aperture size (Fig. 2F–G), but the operculum of other species including *P. michelae* sp. n. is smaller than aperture. The new species is most similar to *P. jentteriana* in its size and smooth shell but differs in colour (*P. jentteriana* has light brown shell and body) and in the morphology of the female gonoduct (in *P. jentteriana* the oviduct loop is wider and includes 3–4 'tube-like evaginations'); the penis of *P. jentteriana* has not been investigated yet (Sitnikova 1991). The new species is similar to young *Parabaikalia elata* (Dybowski, 1975) in the shape of shell and aperture (Fig. 2I), but differs in adult shell size and the length of oviduct loop. *P. elata* is partially sympatric to a new species, they co-occur at sites 3 and 4 (Fig. 1), and thus it was included into molecular analyses (Fig. 6, Table 3).

**Table 3.** Mean (uncorrected) CO1 (lower triangle) and intron ATP α (upper triangle) distances between *P. michelae* sp. n. and other investigated species.

| Taxon | *P. elata* | *P. contabulata* | *P. pulla pulla* | *P. p. tenuicosta* | *P. jentteriana* | *P. zachwatkini* | *P. michelae* sp. n. |
|---|---|---|---|---|---|---|---|
| *P. elata* | | 0.015 | 0.022 | 0.023 | 0.014 | 0.015 | 0.013 |
| *P. contabulata* | 0.057 | | 0.029 | 0.030 | 0.021 | 0.022 | 0.020 |
| *P. pulla pulla* | 0.036 | 0.060 | | 0.010 | 0.010 | 0.011 | 0.013 |
| *P. p. tenuicosta* | 0.036 | 0.055 | 0.006 | | 0.011 | 0.012 | 0.014 |
| *P. jentteriana* | 0.057 | 0.009 | 0.059 | 0.052 | | 0.004 | 0.007 |
| *P. zachwatkini* | 0.039 | 0.050 | 0.040 | 0.038 | 0.047 | | 0.007 |
| *P. michelae* sp. n. | 0.078 | 0.048 | 0.070 | 0.067 | 0.048 | 0.074 | |

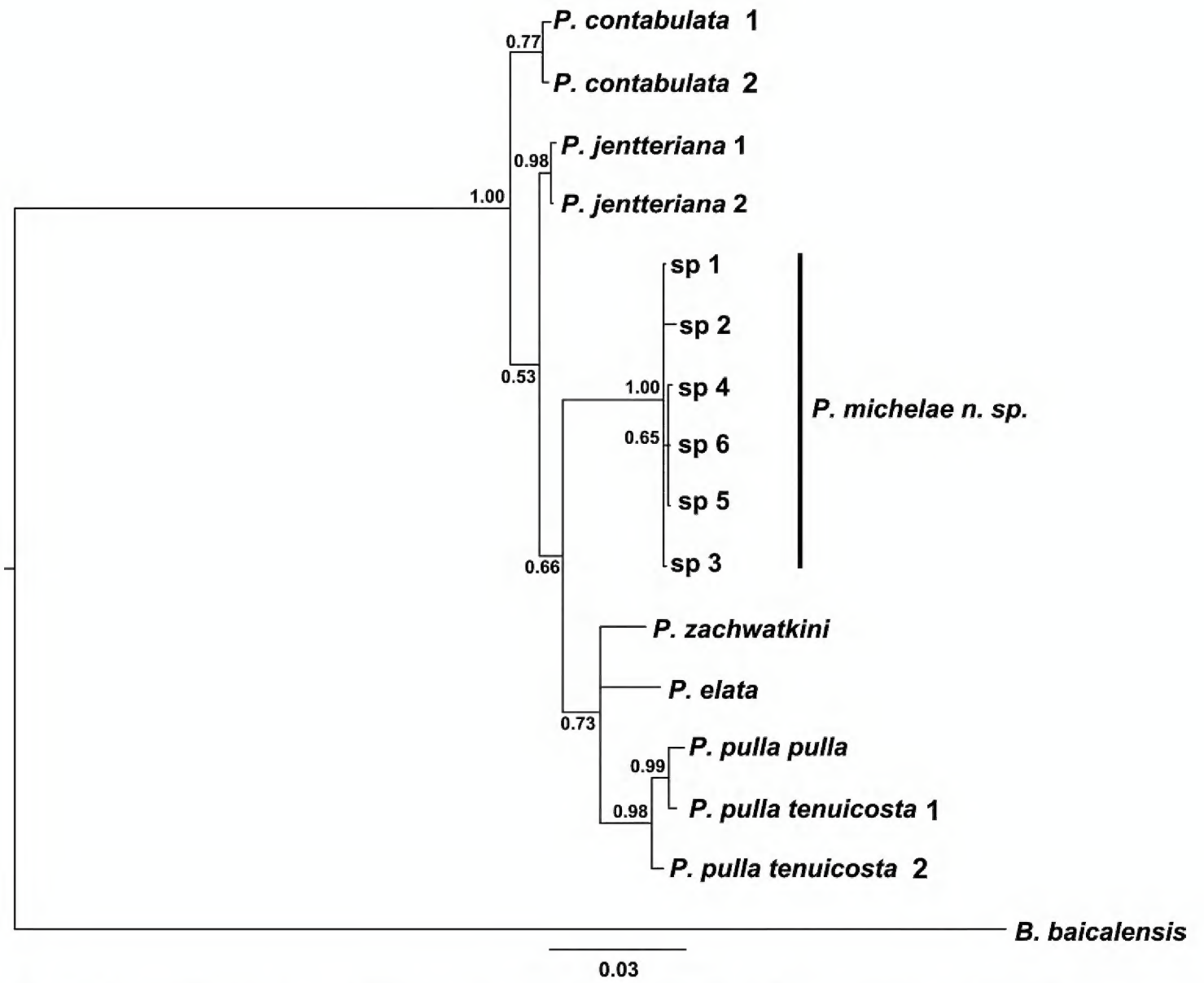


**Figure 6.** Phylogram derived from Bayesian analysis of combined CO1, 16S DNA and ATPase α gene intron sequences. Values at nodes represent posterior probabilities. The scale bar represents the branch length as a measure of substitution per site.

**Molecular phylogeny.** Six specimens of *P. michelae* sp. n. and thirteen specimens of sister species were analyzed using nucleotide sequences from the mitochondrial genes CO1, mtSSU rDNA and nuclear ATPase α intron segment.

JModelTest selected GTR + I + gamma for each CO1 codon positions separately HKY + I for mtSSU rDNA and HKY nucleotide substitution model for intron dataset as the best fit using the Bayesian Information Criterion.

Sequences of *P. michelae* sp. n. cluster in the well-supported clade (posterior probability = 1.00) (Fig. 6). The average mutation distance from the new species to the other *Pseudobaikalia* species was found to be appropriate for species-level distinction: 6.4 ± 1.3%, 2.1 ± 0.2% and 2.1 ± 0.6% for the mitochondrial CO1 and mtSSU rDNA and the nuclear marker, respectively. Interspecies genetic distances are known for other taxa within this family, which can be non-monophyletic.

The lowest mitochondrial genetic distance between *P. contabulata* and *P. jentteriana* are comparable to the distances between representatives of subspecies of *P. pulla*. They both are about 2% for CO1 and 0.03% for mtSSU rDNA. As for the CO1 data, *P. mi-*

*chelae* sp. n. appears to be the sister group to *P. contabulata* and *P. jentteriana* with 4.8% of base substitutions. The minimum genetic distances in case of the intron of *P. michelae* sp. n. is 0.7% to the two species: *P. jentteriana* and *P. zachwatkini* (Table 3). Moreover, there is an important higher-level character difference in the new species sequence profile as all intron sequences of *P. michelae* sp. n. had a relatively large (57 BP) deletion.

## Discussion

The new species is well-differentiated from its sister species in the multigene phylogeny produced here. This is very unusual for Baicaliidae where species with a highly distinctive morphology may be distinguished from each other by very small genetic distances between them. On the other hand, the range of intra-specific polymorphism at least in case of *Baicalia carinata* exceeds the inter-specific distances between several species within this group of gastropods (Zubakov et al. 1997, Peretolchina et al. 2008).

Within *Pseudobaikalia* the interspecific mitochondrial distances are very small (about 1%) in case of *P. jentteriana* and *P. contabulata*. However, these two species are more distant from each other if estimated from the nuclear marker. Most likely it may be explained by secondary and possibly asymmetric hybridization events in the process of their speciation. Similar mechanisms might be responsible for the limited phylogenetic structure based on sequence data for Baicaliidae family as a whole. Thus the genetic separation of *P. michelae* sp. n. from other *Pseudobaikalia* species appears to be an exception.

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## References

Beckman M, Starobogatov YI (1975) Baikal deep-water molluscs and forms related to them. New data of the fauna of Baikal (Part 1). In: Galazi G (Ed.) Trudy Limnologicheskogo Instituta SO AN SSSR. Nauka, Novosibirsk 18: 92–111.

Bouchet P, Rocroi JP, Frýda J, Hausdorf B, Ponder W, Valdés Á, Warén A (2005) Classification and nomenclator of gastropod families. Malacologia. International Journal of Malacology 47: 1–397.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 1–772. doi: 10.1038/nmeth.2109

Doyle JJT, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Dybowski W (1875) Die Gastropoden – Fauna des Baikal-Sees, anatomisch und systematisch bearbeitet. Memories de L'Academie Imperiale des Sciences de St. Petersbourg 22: 1–73.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696–704. doi: 10.1080/10635150390235520

Hausdorf B, Röpstorf P, Riedel F (2003) Relationships and origin of endemic Lake Baikal gastropods (Caenogastropoda: Rissooidea) based on mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 26: 435–443. doi: 10.1016/S1055–7903(02)00365–2

Hershler R, Ponder WF (1998) A review of morphological characters of hydrobioid snails. Smithsonian Contributions to Zoology 60: 1–55. doi: 10.5479/si.00810282.600

Jarman S, Ward RD, Elliott NG (2002) Oligonucleotide primers for PCR amplification of coelomate introns. Marine Biotechnology 4: 347–355. doi: 10.1007/s10126–002–0029–6

Kantor YI, Schileyko AA, Vinarski MV, Sysoyev AV (2010) Continental molluscs of Russia and adjacent territories. Version 2.3.1. http://www.ruthenica.com/categorie-8.html [2 October 2015]

Katana A, Kwiatowski J, Spalik K, Zakrys B (2001) Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. Journal of Phycology 37: 443–451. doi: 10.1046/j.1529–8817.2001.037003443.x

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30: 772–780. doi: 10.1093/molbev/mst010

Kovalenkova MV, Sitnikova TYa, Sherbakov DYu (2015) Genetic and Morphological Diversification in Gastropods of the Baicaliidae Family. Russian Journal of Genetics: Applied Research 5: 110–117. doi: 10.1134/S2079059715020045

Kozhov MM (1936) Molluscs of Lake Baikal — systematics, distribution, ecology, some data on genesis and history. Trudy Baikal'skoi Limnologicheskoi Stantzii 8: 1–350.

Lindholm WA (1909) Die Mollusken des Baikal-Sees (Gastropoda et Pelecopoda), systematisch und zoogeographisch bearbeitet. Wissenschaftliche Ergebnisse einer Zoologischen Expedition nach dem Baikal-See unter Leitung des Professors A. Korotneff in dem Jahren 1900–1902. Zoological studies Lake Baikal 4: 1–106.

Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289–290. doi: 10.1093/bioinformatics/btg412

Peretolchina TE, Sitnikova TYa, Sherbakov DYu (2008) Evolutionary relationships between sister species of endemic gastropods phylum Baicalia (Mollusca, Caenogastropoda). Izvestiya Irkutskogo gosudarstvennogo universiteta [the Bulletin of Irkutsk State University] 1: 67–70.

R Core Team (2015) R: A language and environment for statistical computing. R-Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/ [2 October 2015]

Radoman P (1983) Hydrobioidea a superfamily of Prosobranchia (Gastropoda) (I) Systematics. Serbian Academy of Science and Arts, Monographs 57: 1–256.

Ronquist F, Taslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. doi: 10.1093/sysbio/sys029

Sherbakov DYu (1999) Molecular phylogenetic studies on the origin of biodiversity in lake Baikal. Trends in Ecology and Evolution 14: 99–102. doi: 10.1016/S0169–5347(98)01543–2

Sitnikova TYa (1991) New structure of Baikalian endemic family Baicaliidae (Mollusca, Gastropoda, Pectinibranchia). In: Lynevich A (Ed.) [Morphology and Evolution of Invertebrates]. Nauka, Novosibirsk, 281–295. [In Russian]

Sitnikova TYa, Roepstorf P, Riedel F (2001) Reproduction, duration of embryogenesis, egg capsules and protoconches of gastropods of the family Baicaliidae (Caenogastropoda) endemic to Lake Baikal. Malacologia 43: 59–85.

Sitnikova TYa, Starobogatov YaI, Shirokaya AA, Shibanova IV, Korobkova NV, Adov FV (2004) Gastropoda. In: Timoshkin OA (Ed.) Index of Animal Species Inhabiting Lake Baikal and its Catchment Area. Nauka, Novosibirsk, 937–1002.

Zubakov DYu, Sherbakov DYu, Sitnikova TYa (1997) Phylogeny of the endemic Baicaliidae molluscs inferred from partial nucleotide sequences of the CO1 mitochondrial gene. Molecular Biology (Moscow) 31: 936–939.